## Amendments to the Drawings

The attached four sheets of drawings in Appendix A include changes to Figs. 1-8. These sheets replace the original four sheets including Figs. 1-8.

More specifically, all frames to Figs. 1-8 have been removed as requested by the Examiner, and the references to restriction sties in Figs. 1 and 2 have been adjusted to make them more readable. No changes to the substance of the figures were made.

## Remarks

Claims 1, 3, 7-9, 13, 15, and 17-29 are pending in the present application.

### Specification

Paragraphs [0009], [0052], and [0053] have been amended to correct the informalities pointed out by the Examiner.

Paragraph [0025] has also been amended to align the lines in this paragraph with the rest of the text on the page. Paragraph [0025] has been further amended to clarify that the plant cells are transformed by an expression system that comprises a vector and a DNA construct of the present application inserted into the vector. This amendment is supported by the specification as originally filed, for example, at paragraph [0017].

Paragraphs [0042] and [0047] have been amended to refer to the indicated sequences by their sequence identifier numbers as required by 37 CFR §1.821. A Sequence Listing cofiled with this paper has been added to the specification, which assigns SEQ ID NOs to the sequences appearing in the specification and the claims of the present application. The Sequence Listing includes seven (8) sequences that are fully supported by the specification and claims as originally filed, see page 12, lines 25-28; page 13, lines 1-2; page 15, paragraph [0047], lines 11-12; claim 9, and no new matter has been added by the amendment.

### Drawings

The frames to all drawings have been removed to comply with 37 CFR 1.84(g). The references to restriction sites in Figs. 1 and 2 have been adjusted to become more readable. Each of the corrected drawing sheets has been labeled as "Replacement Sheet" and attached to this paper as Appendix A.

### Claim Objections

Claims 17-18 and 21-23 are objected to because of some formalities in the claims. The applicant respectfully submits that these claims have been amended as suggested by the Examiner.

**35 U.S.C. § 112, Second Paragraph**

Claims 1-29 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as the invention. More specifically, claim 1 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite because (1) it recites "a DNA construct to generate and direct the processing, targeting and stably accumulating of target proteins," (2) it recites "a promoter sequence capable of directing expression," and (3) it is unclear whether the first DNA sequence, second DNA sequence and third DNA sequence are operably linked or just present anywhere in the DNA construct.

Claim 1 has been amended to address each of the indefinite point raised by the Examiner. The applicant respectfully submits that with the amendments, claim1 particularly points out and distinctly claims the subject matter which the applicant regards as one embodiment of the present invention, and is therefore definite.

Claims 4-6 and 16 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in its recitation "derived." Claims 4-6 and 16 have been cancelled. The rejection therefore becomes moot.

Claims 9 and 10 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite in its recitation of "a spacer sequence in front of the transmembrane domain sequence," because, in the Examiner's view, the meets and bounds of the recitation "in front" are unclear. Claim 9 has been amended to clarify that the spacer sequence is "operably linked to" the transmembrance domain sequence. Claim 10 has been cancelled. The applicant respectfully submits that claim 9 is now definite, and the rejection should be withdrawn in view of the amendment.

Claim 15 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite in its recitation "engineered signal peptide" without identifying how the signal peptide sequence is linked to the target sequence. Claim 15 has been amended to clarify that the engineered signal peptide is "operably linked to the first DNA sequence," which as defined in claim 1 is operably linked to the second DNA sequence. Claim 15 has been further amended to require that the signal peptide

sequence is selected from the group consisting of a proaleurain signal peptide, a barley cysteine protease aleurain signal peptide, and a rice storage protein glutelin signal peptide. Therefore, the applicant respectfully submits that claim 15 is now definite, and the rejection should be withdrawn in view of the amendments.

**35 U.S.C. § 112, first paragraph**

Claims 1-29 are rejected under 35 U.S.C. 112, first paragraph, because the Office action finds the claims containing subject matter which was not described in the specification in such a way as to enable any person skilled in the art to make and use the invention commensurate in scope with these claims. Claim 1 has been amended to limit that the second DNA sequence having a transmembrance domain sequence of BP-80 and a cytoplasmic tail sequence of BP-80 or α-TIP. The Office action explicitly admits that such an embodiment is enabled by the specification. Therefore, the applicant respectfully requires the Examiner to withdraw the rejection in view of the amendments to claim 1.

Claim 15 is further rejected because it encompassed any signal peptide sequence that would direct and stably accumulate a target protein in subcompartments of protein storage vacuole of the cells. Claim 15 has been amended to require that the signal peptide sequence is a proaleurain signal peptide, a barley cysteine protease aleurain signal peptide, or a rice storage protein glutelin signal peptide, which is fully enabled by the specification as originally filed. *See* paragraphs [0042] and [0047]. Therefore, the applicant respectfully submits that the subject matter of claim 15 is enabled, and the rejection should be withdrawn in view of the amendment.

Claim 26 is rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement, because of the employment of vector pSB130 or pBI121 in the claimed method. Claim 26 depends from claim 23, which has been amended to clarify the meaning and scope of the method of claim 23. Claim 23 now reads:

> A method for constructing a transgenic plant comprising the steps of:
> a) constructing an expression system comprising a vector and the DNA construct defined as in claim 1, wherein the DNA construct is inserted into the vector;
> b) transforming plant cells with the expression system; and

c) regenerating the transgenic plant from the plant cells to produce the target proteins in seeds of the transgenic plant.

The essence of the method of claim 23 is the DNA construct as defined by claim 1, which is inserted into a vector to construct a plant gene expression system in accordance with at least one embodiment of the present invention. Claim 26 further recites that the vector is pSB130 or pBI 121.

Vectors pBI 121 and pSBB 130 were known to people skilled in the art and readily available to the public when the present application was filed. For example, the binary vector pBI121 is described and used in a research article published in 1999, which is attached in Appendix B. *See* Appendix B, page 292, left column, paragraph 1. The article further refers to Bevan, M., Binary *Agrobacterium* vectors for plant transformation, nucleic Acid Res., 12, 8711-8721 (1984) for the teaching of the binary vector pBI 121. *Id.* at Reference 10. Moreover, PBI 121 has been commercially available from Clontech, Palo Alto, CA before October 2002. *See* Appendix C, page 5436, left column, paragraph 3, lines 10-11. Similarly, the super binary vector pSB 130 was also known to people skilled in the art when the present application was filed. For example, pSB 130 and the method of making the same is described in a doctoral thesis deposited at the library of Yangzhou University in May 2002, which was indexed and available to the public. The relevant part of the thesis was attached in Appendix D. The thesis has also been publicly available online by no later than December 2003. *See* http://ckrd169.cnki.net/grid20/detail.aspx?filename=2003122960.nh&dbname=cdmd2004&filetitle=%e5%9f%ba%e5%9b%a0%e5%b7%a5%e7%a8%8b%e6%8a%80%e6%9c%af%e6%8f%90%e9%ab%98%e7%a8%bb%e7%b1%b3%e8%b5%96%e6%b0%a8%e9%85%b8%e5%90%ab%e9%87%8f, attached as Appendix E.

The method of making an expression system comprising a vector and a certain DNA construct inserted into the vector was also known in the art when the present application was filed. *See* Appendixes B-D. The expression system of the present application can be constructed by inserting the DNA construct of the present application into a vector using a method known in the art and readily available to the public.

Therefore, the applicant respectfully submits that no deposits of biological materials are needed in the present application. The vectors pSB 130 and pBI 121 and the expression systems comprising these vectors and the DNA construct of the present application were enabled by the specification as originally filed.

Claims 1-29 are further rejected under 35 U.S. C. 112, first paragraph, as failing to comply with the written description requirement. Claim 1 has been amended to limit that the second DNA sequence having a transmembrane domain sequence of BP-80 and a cytoplasmic tail sequence of BP-80 or $\alpha$-TIP. The Office action explicitly admits that such an embodiment is supported by the written description of the present application. Therefore, the applicant respectfully requires the Examiner to withdraw the rejection in view of the amendments to claim 1.

**35 U.S.C. § 102 (Novelty)**

Claims 1, 4, 7-9, 11, 13 and 17-20 are rejected under 35 U.S.C. 102(b) as being anticipated by Jiang et al. (The Journal of Cell Biology, 143:1183-1199, 1998, the "Jiang reference").

Claim 1 has been amended to require a seed specific promoter sequence. The Office action admits that the Jiang reference does not teach the use of a seek specific promoter. Therefore, claim 1 is novel over the Jiang reference.

Claims 4 and 11 have been cancelled. Claims 7-9, 13, and 17-20 all depend from claim 1, and therefore they are novel over the Jiang reference at least for the same reason as discussed above for claim 1.

For reasons as described below, claims 1, 7-9, 13, and 17-20 are not obvious over the prior art of record as well.

**35 U.S.C. § 103 (Non-obviousness)**

Claims 2-3, 5-6, 10, 12, 14-16 and 21-29 are rejected under 35 U.S.C. 103(a) as being unpatentable over the Jiang reference in view of Altenbach et al. (Plant Molecular Biology, 13:513-522, 1989, the "Altenbach reference") and Goddijn et al. (Trends Biotechnol. 13:379-387, 1995, the "Goddijn reference"). Claims 2, 5, 6, 10, 12, 14, and 16 have been cancelled. Claims 3, 15, and 21-29 all depend from claim 1, either directly or indirectly.

Claim 1 has been amended to limit the promoter sequence to a "seed-specific" promoter sequence, and specify that the transmembrane domain ("TMD") sequence and the cytoplasmic tail ("CT") sequence are of "BP-80" and "BP-80 or α-TIP," respectfully.

As the Office action admits, the Jiang reference does not teach a DNA construct comprising a seed-specific promoter. The Jiang reference does not teach or suggest that its construct, which does not have a seed-specific promoter, can be expressed in plant seeds. Neither does the Jiang reference teach or suggest that a DNA construct comprising a TMD sequence operably linked to a CT sequence could express a target protein in plant seeds by linking a seed-specific promoter to it. Further, the Jiang reference does not teach or suggest that a seed-specific promoter could function in plant after being linked to the construct as claimed in the current claim 1.

The Office action alleges that the Altenbach reference teaches a seed-specific phaseolin promoter. However, the Altenbach reference only teaches that a construct comprising regulatory regions from a phaseolin gene of French bean can accumulate the Brazil nut methionine-rich protein in tobacco seeds. The regulatory regions include all the sequences of the phaseolin gene except phaseolin coding sequences (nucleotides 75-1852, see page 514, column 2). The Altenbach reference does not teach that the phasolin promoter sequence in the regulatory regions, which is one of the many sequences, is seed-specific and could regulate a foreign gene expression specifically in plant seeds by itself.

Furthermore, the Altenbach reference does not teach that the phaseolin promoter sequence separated from the whole regulatory regions could work in a construct different from that taught by the Altenbach reference, such as in a construct described by the Jiang reference or a construct as claimed in the present application. In fact, there is no teaching or suggestion in the Altenbach reference that the regulatory regions from the phaseolin gene of French bean could direct other constructs to express target proteins in plant seeds.

Therefore, there is no teaching of the use of a seed-specific promoter sequence in the Altenbach reference. And there is no motivation or suggestion in the Jiang reference or the Altenbach reference to modify the construct of the Jiang reference with a seed-specific promoter to achieve a construct as claimed in claim 1 of the present application.

The Goddijn reference allegedly teaches that it is well known in the art that seeds can be used as "bioreactors" for the production of pharmaceutically or industrially important products. However, the Goddijn reference does not teach a seed-specific promoter sequence or the use thereof in a DNA construct. Further, the general teaching that seeds can be used as "bioreactors" does not provide the specific motivation or suggestion required to modify the construct of the Jiang reference with a seed-specific promoter to obtain a DNA construct as claimed by claim 1 of the present application.

Therefore, claim 1 is not obvious over the Jiang reference in view of the Altenbach reference and the Goddijn reference.

All other pending claims (3, 7-9, 13, 15, and 17-29) are dependent on claim 1, either directly or indirectly. Therefore, they are patentable over the Jiang reference in view of the Altenbach reference and the Goddijn reference at least for the same reason as for claim 1.

## Conclusion

In light of the above amendments and remarks, the Examiner is respectfully requested to reconsider the present application, withdraw the rejections, and prepare a Notice of Allowability allowing all the pending claims (1, 3, 7-9, 13, 15, and 17-29).

The Commissioner is hereby authorized to charge any additional fees which are presently required, or credit any overpayment, to Deposit Account No. 13-0017.

Respectfully submitted,

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Yufeng Ma
Reg. No. 56,975
Attorney for Applicants

McAndrews, Held & Malloy, Ltd.
500 W. Madison, 34th Floor
Chicago, IL 60661
Telephone: (312) 775-8000
Facsimile: (312) 775-8100

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Research Article

# *Agrobacterium tumefaciens*-Mediated Transformation of Sesame (*Sesamum indicum* L.)

Kemal Melih TAŞKIN
Akdeniz University, Graduate School of Natural and Applied Sciences, Department of Field Crops, Antalya–TURKEY
A. Gülhan ERCAN
Akdeniz University, Graduate School of Natural and Applied Sciences, Department of Field Crops, Antalya–TURKEY
Kenan TURGUT
Akdeniz University, Faculty of Agriculture, Department of Field Crops, Antalya–TURKEY

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**Abstract:** *Sesamum indicum* L. cv. Özberk was infected with various *Agrobacterium tumefaciens* strains to screen susceptibility to infection by Agrobacteria. Following infection, tumorigenesis was efficient with wild type *A. tumefaciens* strains, A281 and A136 NC, and transgenic cells were obtained with disarmed *A. tumefaciens* strain LBA4404/pBI121, harbouring a reporter gene. At the wound sites, tumorigenesis induced by the succinamopine strain A281 was more extensive than by the octopine strain A136 NC.

**Key Words:** *Sesamum indicum*, *Agrobacterium tumefaciens*, virulence, transformation.

## Susam (*Sesamum indicum* L.) Bitkinin *Agrobacterium tumefaciens* Aracılığı ile Transformasyonu

**Özet:** *Sesamum indicum* L. cv. Özberk *Agrobacterium*'a duyarlılığının belirlenmesi amacıyla çeşitli *Agrobacterium tumefaciens* suşları ile enfekte edilmiştir. Enfeksiyonu takiben, yabanıl tip *A. tumefaciens* suşları A281 ve A136 NC tümör indüklerken, reporter gen taşıyan disarmed *A. tumefaciens* suşu LBA4404/pBI121, transgenik hücreler oluşturmuştur. Yaralı bölgelerde succinamopin suşu A281 tarafından indüklenen tümörler, octopin suşu A136 NC tarafından indüklenen tümörlerden daha büyük olmuştur.

**Anahtar Sözcükler:** *Sesamum indicum*, *Agrobacterium tumefaciens*, virülentlik, transformasyon.

## Introduction

*Sesamum indicum* L. *(Pedaliaceae)* is grown as an oil seed crop because sesame oil contains high percentages of linoleic and oleic fatty acids, but mostly used as food in Turkey. However, there are some problems limiting sesame production in Turkey. These are plant diseases, indeterminate flowering and dehiscent capsules. Plant diseases such as *Alternaria sesemicola* Kaw., *Oxysporum* Schlectend.: Fr., and *Phytophora parasitica* Dastur cause considerable damage and reduce the yield. Indeterminate flowering and dehiscent capsules are the most important factors limiting sesame production. Conventional plant breeding methods have produced some improvement but not enough. Therefore, new technologies such as biotechnology should be introduced to develop new varieties. Gene transfer via *Agrobacterium tumefaciens* is one of the useful technique to overcome such problems. Wild-type, oncogenic strains of *A. tumefaciens* harbour large plasmids called Ti (Tumor-inducing) plasmids, which contains several genes important for tumorigenicity (1). It is known that *A. tumefaciens* strains can be used to transfer individual genes of interest together with the Ti T-DNA, into the genome of plants. Successful foreign gene transfer to a plant was first reported by Zambryski et al. (2) using genetically manipulated strains of *A. tumefaciens.* According to host range studies (3) *Agrobacterium* can infect a wide spectrum of dicots and some monocots. Recently in vitro regeneration (4), in vitro propagation (5), shoot tip culture (6), protoplast culture (7) and naphthaquinone production (8) of sesame have been reported. However, the susceptibility of *S. indicum* to infection by *Agrobacteria* has not been reported. In this study, our aim was to determine the virulence of various wild type oncogenic *A. tumefaciens* strains against *S. indicum* and gene transfer to sesame using disarmed *A. tumefaciens* strains carrying a reporter gene.

## Materials and Methods

### Bacterial Strains

Six wild-type oncogenic (Table 1) and two disarmed (LBA4404/pBI121, pGV 2260/p35S-*GUSINT*) *A. tumefaciens* strains (obtained from Dr. Rod Scott, Bath University, UK) were used for transformation experiments. Octopine type disarmed strain LBA4404 (9) carries the binary vector pBI121 (10) and non-oncogenic vir helper plasmid. The plasmid pBI121 contains a nopaline synthase promoter sequence in front of a neomycin phosphotransferase II *(NPTII)* gene which confers resistance to kanamycin and a *GUS (β-glucuronidase)* gene driven by CaMV 35S promoter. Disarmed strain pGV2260 (11) carries plasmid p35S *GUS INT* (12). The plasmid p35S *GUS INT* contains the coding sequence of the *NPT II* gene under the control of nopaline synthase (NOS) promoter, and the *GUS* gene is under the control of the CaMV 35S promoter. In the p35S *GUS INT* construct, the *GUS* gene is interrupted by a plant intron and shows activity only in transformed plant cells. All the strains were grown in semi-solid Nutrient Agar (NA) or liquid Nutrient Broth (in a shaker at 150 rpm, Nuve SL350) media for 48 hours at 28 °C. NA medium contains 5 g/l peptone, 5 g/l sodium chloride, 1.5 g/l beef extract, 1.5 g/l yeast extract, 15 g/l agar and pH 7.4. NB medium contains 3 g/l bacto beef extract, 5 g/l bacto peptone and pH 6.8.

### Plant Growth and Inoculation

Seeds were planted in 10 cm pots consisting of peat:perlite (1:1). Plants were grown in a growth chamber with 18 h photoperiod at 25 °C. Twelve weeks after germination, sesame plants (mature plants, during capsule growth) were inoculated with wild type *A. tumefaciens* strains. Bacterial inoculum was prepared by culturing each strain on semi-solid NA medium for 2 days at 28 °C. A syringe needle was used to scratch the stem about 2 mm deep and 0.5 mm wide for a length of 5 mm in 3 parallel lines at each internode (3 internodes per plant). A minimum of 3 plants per strain were used. The wound site was filled with bacteria (13, 14). Tumor formation was scored regularly for up to 2 months. Tumor frequency or the percentage of inoculated wounds that developed tumors as well as tumor size were monitored daily and scored.

### Endogenous Level of Kanamycin Resistance of Plant Material

Kanamycin is usually used as a selection agent for transformation systems. For this reason, susceptibility of sesame to kanamycin was determined. The surface sterilized seeds (4) were aseptically placed in plates (9 cm petri dishes) containing MS medium (30 ml) supplemented with 0.8% agar (w/v), 3% sucrose (w/v) and 0, 50, 100 or 150 mg/l kanamycin for germination. The pH was adjusted to 5.7. Four repetitions and a minimum of 100 seeds were used per kanamycin concentration. Seedlings were grown at 25 °C with a 16 h photoperiod, under white fluorescent light.

Table 1. Tumors Induced by Wild-Type *A. tumefaciens* Strains After One Month Inoculation of Sasame Plants In Vivo.

| Wild Type | *A. tumefaciens* Strains | Tumor induction Frequency (%) |
|---|---|---|
| A281 | (Succinamopine) | 54 |
| A136NC | (Octopine) | 50 |
| T37 | (Nopaline) | - |
| A6 | (Octopine) | - |
| C58 | (Nopaline) | - |
| ACH5 | (Octopine) | - |

### In Vitro Transformation of Cotyledon Explants

Sesame seeds were surface sterilized (4) and then sown in 9 cm petri dishes containing 30 ml of Murashige & Skoog (MS) medium (15) supplemented with 0.8% (w/v) agar and 3% sucrose (w/v), pH 5.7. Seeds were germinated at 25 °C with a 16 h photoperiod, under white fluorescent light (3000 lux). Seven days after germination, cotyledons were excised and inoculated with *Agrobacterium* strains. Overnight bacterial cultures were grown in 25 ml NB medium at 28 °C in an orbital shaker (150 rpm). For disarmed strains, NB medium was supplemented with 50 µg/l kanamycin and rifampicin antibiotics. Cotyledon explants were immersed in overnight bacterial cultures diluted 1:100 (v/v) in 25 ml liquid MS medium containing 3% sucrose for 10 minutes then transferred to co-cultivation medium (semi-solid MS medium without antibiotics) for 2 days. After co-cultivation, cotyledon explants were transferred to MS medium supplemented with 0.8% (w/v) agar, 3% sucrose (w/v) 50 mg/l kanamycin as a selective agent and 400 mg/l augmentin to kill the bacteria. This medium also included 0.1 mg/l NAA + 8 mg/l BAP. Control explants were dipped into liquid MS medium without bacteria. Inoculations were carried out in a laminar flow hood. A minimum of 100 explants per strain were used. They were subcultured every 2 to 3 weeks.

### Histochemical *GUS* Assay

Transgenic cells were confirmed by histochemical *GUS* assay (16). For analysis, 5 mg X-Gluc was dissolved in 100 µl dimethylformamide and total volume was

increased to 10 ml with 50 mM NaPO4 pH 7. Callus regenerated from co-cultivated explants were then treated with this solution at 37 °C for 72 hours. Finally, the reaction was stopped with 70% ethanol.

## Results

### Inoculations With Wild-type *A. tumefaciens* Strains

The response of sesame plants to strains containing wild-type Ti-plasmids are given in Table 1. Following inoculation, tumor formation was distinguishable within 2 weeks. Among the wild types, only two strains (A281 and A136 NC) induced tumors on sesame plants. The succinamopine strain A281 induced tumors at a frequency of 54% at inoculated sites on the 3 plants (Figure 1a). The octopine strain A136 NC induced tumors at a frequency of 50% at inoculation sites on the 3 plants. Furthermore, succinamopine tumors were larger than octopine tumors (average 0.2 mm and 0.1 mm respectively). However, the other wild type strains (ACH5, T37, A6, C58) were infective on sesame (Table 1) under the experimental conditions.

### Determination of Kanamycin Resistance

Sesame seeds were germinated on MS media containing different concentrations of kanamycin and without kanamycin (control). After 10 days, in control plants (without kanamycin), all the seeds germinated and looked healthy. However, in the other MS media containing kanamycin, germination frequency was found to be 94% (50 mg/l kanamycin), 88% (100 mg/l kanamycin) and 80% (150 mg/l kanamycin). After 3 weeks of culture on MS medium supplemented with varying amounts of kanamycin, seedlings were bleached and dead regardless of kanamycin level. Thus, 50 mg/l kanamycin appeared to be convenient for selection.

### Co-cultivation of Cotyledon Explants with *A. tumefacines* Strains

After exposure to wild-type *A. tumefaciens* strains and planting on hormone-free MS medium containing 400 mg/l augmentin, some cotyledons showed tumor formation. But only two strains (A281 and A136 NC) induced tumors (Figure 1b). These tumors covered the whole explants in two weeks of culture.

Following co-cultivation with disarmed *Agrobacterium* strains (LBA 4404/pBI121 and pGV 2260/ p35S *GUSINT*), cotyledon explants produced callus on regeneration medium (4) supplemented with kanamycin (50 mg/l) and augmentin (400 mg/l). However, for explants treated with liquid MS medium (control), callus formation did not occur on the same regeneration medium. Thus, histochemical *GUS* assay was performed with these calli to determine *GUS* activity. However, only the explants co-cultivated with LBA 4404/ pBI 121 strain revealed *GUS* gene expression (Figure 1c) and gave 4-5 blue spots on each cotyledon explant. However, transgenic shoots were not obtained in this study. In preliminary experiments, different dilutions of overnight bacterial cultures (1:10, 1:25, 1:50 and 1:100) were tested in order to improve transformation efficiency. However, generally more necrosis on cotyledon explants was seen at the highest concentrations of bacteria.

## Discussion

This study presents the first report on the susceptibility of a sesame cultivar to *A. tumefaciens.* Although there is one report on production of naphthaquinone by a hairy root culture induced by direct inoculation of *S. indicum* via *A. rhizogenes* ATCC 15834 (8) there is no report on the susceptibility of sesame to *A. tumefaciens* infection.

In this study, A281 resulted in larger tumor formation than A136 NC on sesame plants. The strain A281 is known as a broad host-range supervirulent strain (17) and has been very useful for transforming recalcitrant species. Davis et al. (18) reported that tumors induced by A281 strain were larger than other wild type strains on cotyledon explants of tomato. These reports support the results of the present study.

Recently in vitro regeneration (4), in vitro propagation (5) shoot tip culture (6), protoplast culture of sesame (7) have been reported. Taşkın and Turgut (4) obtained adventitious shoot regeneration which is important for *A. tumefaciens* mediated gene transfer systems. For this reason, this regeneration system was used for transformation studies. However, co-cultivation of cotyledon explants with disarmed *Agrobacterium* strains reduced regeneration efficiency over non-transformed controls. The reason for this reduction was not investigated further but may be related to the hypersensitive response of sesame explants to *A. tumefaciens* infections. This result is consistent with that of Orlikowska et al. (19). They described the characterization of factors affecting *A. tumefaciens*-mediated transformation of safflower seedlings explants and reported that hypersensitive response to bacterial infection may reduce organogenetic potential furthermore, negative effects of antibiotics (kanamycin) on regeneration were observed for safflower (19).

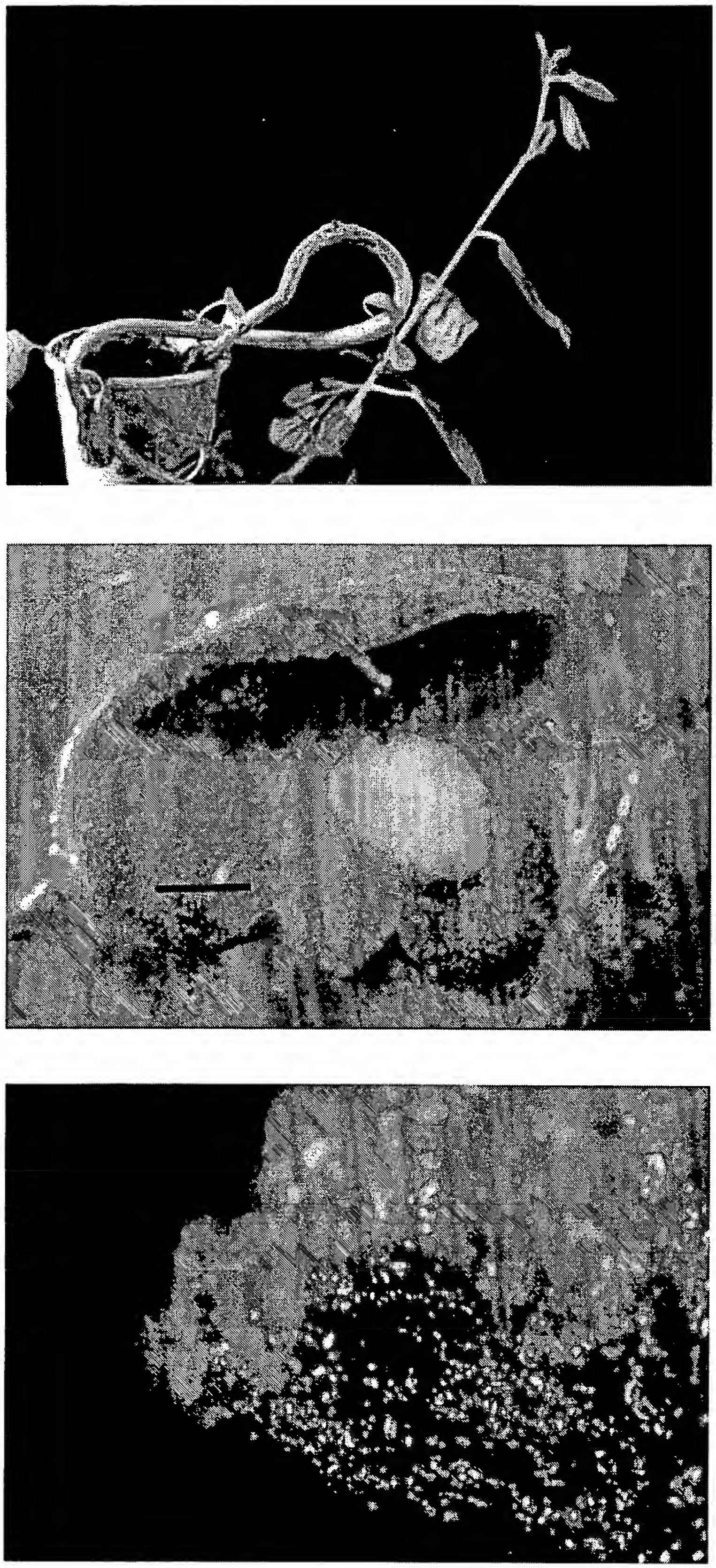

Figure 1 a) Tumors induced by wild type *A. tumefaciens* strain A281 after one month inoculation of sesame plants in vivo. b) Tumor induced by *A. tumefaciens* strain A136 NC on cotyledon explants of sesame after one week in vitro. c) Callus regenerated after co-cultivation with disarmed strain LBA 4404/pBI 121 in vitro showed stable *GUS* gene expression.

Therefore, it may be useful to try different antibiotic selection systems.

The results of this work demonstrate that sesame is susceptible to wild–type *Agrobacterium* strains A281 and A136 NC. Furthermore, it is possible to obtain stable T-DNA transfer by inoculating cotyledon explants with disarmed strain LBA 4404/ pBI121, although this occurs at a low frequency. However, no shoot regeneration was obtained in this study due to the low regeneration ability of sesame (4). Alternatively, it may be possible to obtain better regeneration systems from other genotypes. In conclusion, disarmed *Agrobacterium* strains based on wild-type oncogenic A281 or LBA4404/ pBI 121 could be used to obtain transgenic sesame plants.

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# Production of FaeG, the Major Subunit of K88 Fimbriae, in Transgenic Tobacco Plants and Its Immunogenicity in Mice

Yahong Huang,[1,2] Wanqi Liang,[2] Aihu Pan,[2] Zhiai Zhou,[3] Cheng Huang,[1] Jianxiu Chen,[1] and Dabing Zhang[2]*

*The Department of Biological Science and Technology, School of Life Science of Nanjing University, Nanjing,[1] and Shanghai Key Laboratory of Agricultural Genetics and Breeding, Agro-biotech Research Center,[2] and Shanghai Key Laboratory of Agricultural Genetics and Breeding, The Institute of Animal and Veterinary Sciences,[3] Shanghai Academy of Agricultural Sciences, Shanghai, People's Republic of China*

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**Transgenic tobacco plants stably expressing recombinant FaeG, which is the major subunit and adhesin of K88ad fimbriae, were obtained. Analysis of sera from immunized mice indicates that in mice, the immunogenicity induced by plant-derived FaeG protein is comparable to that generated with traditional approaches.**

Enterotoxigenic *Escherichia coli* (ETEC) strains are commonly associated with neonatal diarrhea in piglets (6, 11, 12, 13, 19). Among the different ETEC strains, those expressing K88 fimbrial antigen are the most prevalent type (1, 18). Fimbriae (or pili) are the primary pathogenicity factors of this bacterium and are responsible for its adhesion to enterocyte receptors. Analysis of the genetic organization of the K88 gene cluster has revealed that at least eight structural genes are involved in biosynthesis. FaeG (27.6 kDa) is the so-called major fimbrial subunit protein that also carries the adhesive properties of the K88 fimbriae (4, 17, 24, 26).

Vaccines containing purified K88 fimbriae, formalin-inactivated ETEC, or engineered *E. coli* expressing K88 fimbriae have been used to vaccinate pregnant sows, and passive transfer of lacteal immunity from the vaccinated dams can protect piglets from ETEC infection (5, 10, 15, 21, 25, 27). Although proven effective for the prevention of disease, limiting the widespread use of these vaccines are the fact that bacteria might not be inactivated fully and the high cost of producing and preserving these vaccines.

Recently, the use of plants as bioreactors has become of special interest, as they allow production of recombinant proteins in large quantities at relatively low cost (14, 22, 23, 30). For the development of FaeG-based vaccine against K88 ETEC strains in plants, we constructed pBI8801, a plant binary expression vector containing the K88ad fimbrial major antigen gene (*faeG*). First, p8801 (31), a parental plasmid which contains *faeG* without a signal peptide coding region, was digested with *Bam*HI and *Sac*I and the 789-bp *faeG* fragment was cloned into the digested plant expression vector pBI 121 (Clontech, Palo Alto, Calif.). This led to the creation of pBI8801, a binary vector with a cauliflower mosaic virus (CaMV 35s) promoter and a nopaline synthase terminator. Triparental matings were then performed as described by Ditta et al. (7) to transfer plasmid pBI8801 into *Agrobacterium tumefaciens* LBA4404.

Thereafter, transgenic tobacco (*Nicotiana tabacum*) was obtained by a modified leaf-disk cocultivation method using *A. tumefaciens* harboring pBI8801 (16). PCR analysis was carried out to show the presence of an amplified product of the expected size (789 bp) in the genomic DNAs (8) of tested kanamycin-resistant plants (data not shown). Reverse transcriptase PCR (RT-PCR) was used according to the protocol of a PowerScript RT kit (Clontech) to test whether *faeG* was transcribed in the transgenic plants; a fragment with expected size was observed for all the tested transformants, whereas no product was observed for nontransgenic plants (Fig. 1A).

The transgenic plants were further tested for transcriptional activity by examining the expression of *faeG* at translational level. Total soluble protein (TSP) was obtained from plant leaves following the method described by Arakawa et al. (2). The presence of the recombinant protein in the transformants harboring *faeG* was investigated by immunoblot analysis using the anti-FaeG serum (1:300) (31) to probe recombinant protein and an alkaline phosphatase-conjugated anti-rabbit immunoglobulin G (IgG) goat antiserum (Promega, Madison, Wis.) (1:5,000) as the second antibody. There were clear blotting bands with a molecular mass of 27.6 kDa in the tested transgenic plants (Fig. 1B), and no cross-reaction with the anti-FaeG serum was observed in nontransgenic plants. Then, Western blot analysis (28) and an enzyme-linked immunosorbent assay (2) were used to quantify the expression level of the recombinant protein in the transgenic plants. Results of these measurements indicated that recombinant FaeG protein constituted approximately 0.15% of the TSP in a highly expressed transgenic tobacco plant.

It has been shown previously that FaeG can be rapidly degraded without the aid of the chaperone molecule (FaeE) in the host ETEC (3). To evaluate recombinant FaeG expression level variations and the stability of expression levels in the different generations of transgenic tobacco plants, the leaves from the first, second, and third generations ($T_0$, $T_1$, and $T_2$, respectively) of two transgenic plant lines were harvested for immunological analysis. Results indicated that the expression levels among the three generations did not differ greatly (Fig. 2). The little variation observed was likely due to a lack of some

* Corresponding author. Mailing address: Shanghai Key Laboratory of Agricultural Genetics and Breeding, Agro-Biotech Research Center, Shanghai Academy of Agricultural Sciences, 2901 Beidi Rd., Shanghai 201106, People's Republic of China. Phone and Fax: 0086-21-62208750. E-mail: zdb30@hotmail.com.

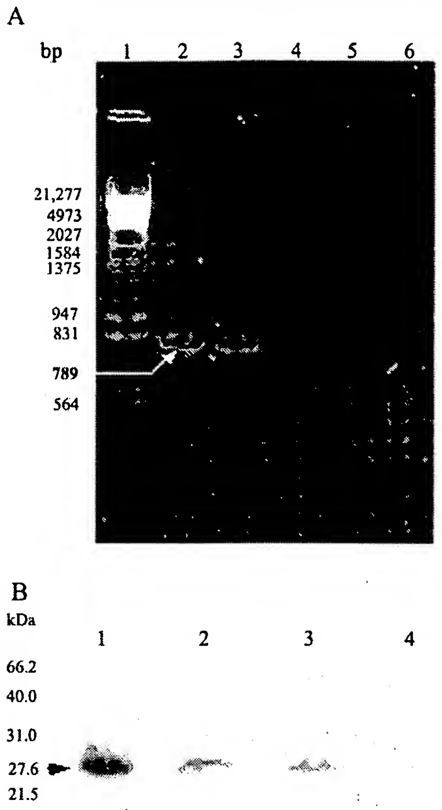


FIG. 1. Analysis of the expression of *faeG* in transgenic plants. (A) Detection (using RT-PCR) of *faeG* transcription in transgenic plants. RT-PCR was performed (using specific primers that amplify a 789-bp DNA fragment of *faeG*) with total RNA from leaves of transgenic tobaccos (32). Lane 1, λDNA (digested with *Hin*dIII/*Eco*RI) molecular weight marker; lanes 2 and 3, RNA from transgenic tobacco; lanes 4 and 5, PCR amplification without RT reaction as a control for DNA contamination; lane 6, RNA from nontransgenic tobacco. (B) Immunoblot detection of recombinant FaeG synthesized in transgenic tobacco. TSP from tobacco leaves was fractionated by sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis. The recombinant FaeG protein was detected with anti-FaeG antibody as the primary antibody and alkaline phosphatase-conjugated goat anti-rabbit IgG as the secondary antibody. Lane 1, 0.5 μg of purified recombinant FaeG expressed in *E. coli* $BL_{21}(DE_3+K88)$ as a positive control; lanes 2 and 3, 100 μg each of TSP from transgenic tobacco plants; lane 4, 100 μg of TSP from nontransgenic tobacco.

related protease specific for the digestion of FaeG in plants. Alternatively, within the plant cell there might be some chaperone-like proteins just like FaeE in K88ad ETEC (3) that may serve to stabilize FaeG and prevent its degradation by the protease.

Since one of the ultimate purposes of this study was to generate recombinant protein with immunogenicity, a group of 24 adult (60- to 90-day-old) female KM mice (purchased from Shanghai Laboratory Animal Center) were immunized intraperitoneally on days 0, 14, 28, and 42 with 0.5-ml transgenic tobacco leaf extracts (containing 7.5 μg of recombinant FaeG per animal per injection) emulsified in an identical volume of complete or incomplete Freund's adjuvant. Mice were bled before each injection. After the last inoculation, the immunized mice were also bled at weekly intervals over a period of 5 weeks. With the same immunizing schedule, each of a group of six KM mice was immunized with nontransgenic plant leaf extracts as a negative control. Moreover, each of a group of six KM mice was immunized with 7.5 μg of purified K88ad fimbriae as a positive control.

By a method described previously (29), K88ad fimbrial antigens were isolated from *E. coli* strain $C_{83902}$ (a standard ETEC strain expressing K88ad fimbriae as determined by the China Institute of Veterinary Drug Control). With purified recombinant FaeG expressed in *E. coli* $BL_{21}(DE_3+K88)$ as an antigen (31), an enzyme-linked immunosorbent assay was performed as described by Arakawa et al. (2) to detect specific serum antibodies. Alkaline phosphatase-conjugated anti-mouse goat IgG (Sigma, St. Louis, Mo.) (1:10,000) was used as the second antibody. The reaction was developed by the addition of the substrate nitroblue tetrazolium–5-bromo-4-chloro-3-indolylphosphate and read at 405 nm in a universal microplate reader (Bio-Tek). The antibody titer was defined as the $\log_{10}$ reciprocal of the highest serum dilution that consistently presented (in at least three consecutive independent determinations) readings of optical density at 405 nm of 3 standard deviations over the mean optical density of the sera from six mice immunized with nontransgenic tobacco plants.

As shown in Fig. 3A the recombinant FaeG produced by transgenic plants can elicit a specific antibody response. Specific anti-FaeG antibodies were first detected in the immunized mice 3 weeks after the first antigen injection. The immunized mice developed a serum antibody response that peaked at $10^4$ 1 week after the last inoculation and then declined over the next 3 weeks. Mice immunized with the leaf extract from nontransgenic plants showed no immune response. The immune response of mice immunized with transgenic plant extracts was similar to that of mice immunized with purified K88ad fimbriae (Fig. 3A).

The specificity of this anti-FaeG response was also confirmed by immunoblot analysis, using the purified K88ad fimbriae and recombinant FaeG from *E. coli* $BL_{21}(DE_3+K88)$ as standard antigens. Sera (diluted 1:50) from mice immunized with transgenic plants expressing recombinant protein specifically recognized proteins with relative mobility identical to that of the protein recognized by the serum raised against bacterium-derived FaeG (Fig. 3B, lanes 1, 2, 3, and 4), whereas no immunological cross-reaction with either purified K88ad fimbriae or recombinant FaeG in serum from the mice immunized with nontransgenic plant leaf extracts was observed (Fig. 3B, lanes 5 and 6).

To test whether the sera from the mice immunized with

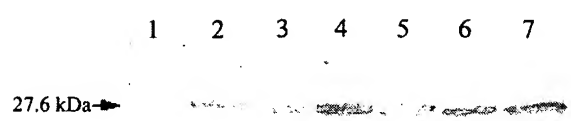


FIG. 2. Immunoblot analysis of the expression level of recombinant FaeG in $T_0$, $T_1$, and $T_2$ generations of transgenic tobacco plants. A total of 100 μg of TSP was loaded for each lane. Lane 1, a nontransgenic plant; lanes 2 and 3, $T_0$ transgenic plants; lanes 4 and 5, $T_1$ transgenic plants; lanes 6 and 7, $T_2$ transgenic plants.

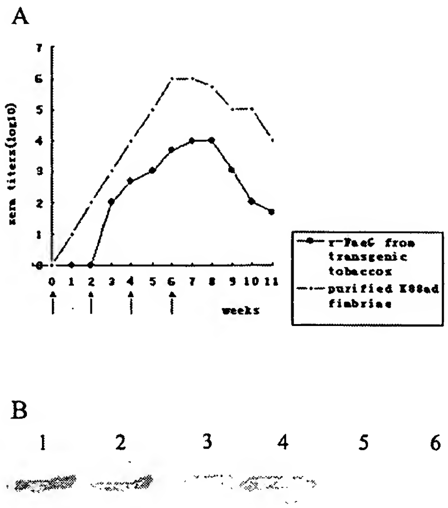


FIG. 3. Immunogenicity of recombinant FaeG from transgenic tobacco. (A) Mice were immunized intraperitoneally on days 0, 14, 28, and 42 with either purified K88ad fimbriae or crude extracts from transgenic tobacco leaves. Arrows indicate the inoculating schedule. (B) Anti-FaeG antibodies detected by immunoblot analysis. ETEC K88ad fimbriae and purified recombinant FaeG from *E. coli* $BL_{21}$ ($DE_3$+K88) were loaded in a well for sodium dodecyl sulfate–15% polyacrylamide gel electrophoresis and transferred to nitrocellulose membrane. After the reaction mixtures were incubated with the sera of mice immunized with purified fimbriae, transgenic tobacco leaf extracts, or nontransgenic tobacco leaf extracts and then washed three more times, the reactions were developed by the addition of the substrate nitroblue tetrazolium–5-bromo-4-chloro-3-indolylphosphate. Lanes 1 and 2, serum from a mouse immunized with purified fimbriae as a positive control; lanes 3 and 4, serum from a mouse immunized with transgenic tobacco extracts; lanes 5 and 6, serum from a mouse immunized with nontransgenic tobacco extracts as a negative control. Lanes 1, 3, and 5, 0.5 μg of purified K88ad fimbriae; lanes 2, 4, and 6, 0.5 μg of purified recombinant FaeG from *E. coli* $BL_{21}$($DE_3$+K88).

transgenic plant extract could interact with the K88ad ETEC, a slide agglutination assay was performed as previously described (20). Sera from the mice immunized with transgenic tobacco at a dilution of 1:20 agglutinated strain $C_{83902}$. In contrast, the use of the sera from the mice immunized with nontransgenic tobacco extract resulted in no agglutination.

The fact that sera from mice immunized with leaf extracts from transgenic plants can cause the agglutination of K88ad ETEC suggests that the same sera can also be effective in mitigating relevant diarrhea symptoms. To this end, ligated ileal loops from rabbits were used to test whether the sera could reduce the adherence of K88ad fimbria-expressing ETEC and inhibit the expression of enterotoxin in this strain in vivo. Rabbit ileal loop studies were performed with 3-kg-body-weight male New Zealand White rabbits as previously described (9). Rabbits were subjected to fasting for 24 h prior to surgery. Laparotomy was performed to externalize the intestine by aseptic technique under anesthesia with intramuscularly administered Sumianxin (purchased from the Quartermaster University of the People's Liberation Army) (0.2 ml/kg). Loops were created in the jejunum by placing ligatures at 6- to 7-cm intervals and separating loops with a 0.5- to 0.6-cm interposing loop. $C_{83902}$ strains were grown from single colonies in 5 ml of Luria-Bertani medium, and the number of cells was adjusted to approximately $10^9$ CFU/ml, with each aliquot containing 500 μl of $C_{83902}$. Serum (100 μl) from a mouse immunized intraperitoneally with transgenic tobacco or nontransgenic tobacco was added to ETEC aliquots to neutralize K88ad-expressing ETEC, and the mixture was incubated for 12 h at room temperature. Then, the 0.6-ml mixture was injected into each loop in random fashion, the intestine was internalized, and the incision was closed. After 18 h of incubation, the consecutive ileal loops were excised, the weights of the loops were measured, and then the loops were punctured to permit measurement of the weight of the empty loops for determination of the significance of fluid reduction in the sera from the mice immunized with transgenic plant extracts. The volume of fluid accumulated in ileal loops inoculated with $C_{83902}$ treated with the sera from the mice immunized with transgenic or nontransgenic plant extracts was measured and expressed as the ratio *V/L* (volume [*V*] [in microliters]/loop length [*L*] [in centimeters]) (Table 1).

As shown in Table 1, the mean values of *V/L* for each group were 63.61 ± 22.32 (mean ± standard error of the mean) for the mice immunized with transgenic tobacco extracts and 154.48 ± 15.11 (mean ± standard error of the mean) for the mice immunized with nontransgenic tobacco extracts. A Student's *t* test revealed significant fluid reduction in comparisons between the sera from the mice immunized with transgenic tobacco plants extracts and the sera from the mice immunized with nontransgenic tobacco extracts ($P < 0.05$). Moreover, ileal loops injected with physiological saline solution did not show significant accumulation of fluid (Table 1) or increased volume (data not shown), suggesting that there were no inflammatory responses caused by mechanical disturbances during loop ligation. This result implies that mouse sera stimulated by plant-derived recombinant FaeG can neutralize K88ad fimbria-expressing ETEC in vivo.

The results of this work suggest the possibility of producing

TABLE 1. Parameters of rabbit ileal loop ligation analysis[a]

| Sample[b] | Loop length (cm) | Total weight of ileal loop (g) | Weight of empty ileal loop (g) | Vol of accumulated fluid (ml) | *V/L* |
|---|---|---|---|---|---|
| A | 6.55 ± 0.07 | 2.803 ± 0.025 | 1.206 ± 0.093 | 1.19 ± 0.01 | 181.70 ± 4.12 |
| B | 6.32 ± 0.47 | 2.107 ± 0.213 | 1.107 ± 0.178 | 0.97 ± 0.12 | 154.48 ± 15.11 |
| C | 6.68 ± 0.58 | 1.834 ± 0.434 | 1.349 ± 0.320 | 0.55 ± 0.29 | 63.61 ± 22.32 |
| D | 6.50 ± 0.28 | 2.175 ± 0.182 | 1.850 ± 0.071 | 0.38 ± 0.11 | 56.31 ± 15.35 |

[a] The ratio of accumulated fluid volume to loop length (*V/L* [in micrograms per centimeter]) was used to express the serum-neutralized effect on K88ad ETEC in ileal loop ligation as previously described (9). A significant difference in neutralizing K88ad ETEC in the sera from the mice immunized with plant-expressed recombinant FaeG was observed in comparison with the sera from the mice immunized with nontransgenic tobacco extracts.

[b] A, K88ad ETEC not treated by sera; B, K88ad ETEC treated with the sera of mice immunized with nontransgenic plant leaf extract; C, K88ad ETEC treated with the sera of mice immunized with transgenic plant leaf extract; D, physiological saline solution instead of K88ad ETEC.

a new alternative vaccine for diarrhea of piglets. Compared to traditional vaccines, this alternative vaccine will be less expensive and more convenient to store. To our knowledge, this is the first report to demonstrate that FaeG can be synthesized in plants. This work also provides a model for the use of plants for the production of vaccines against other ETEC with proteinaceous fimbriae or pili.

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# 学　位　论　文

# 基因工程技术提高稻米赖氨酸含量

# Genetically Engineering Rice for Increased Lysine


刘　巧　泉

指导教师姓名：顾铭洪　教授　扬州大学　江苏扬州
辛世文　教授　香港中文大学　香港新界


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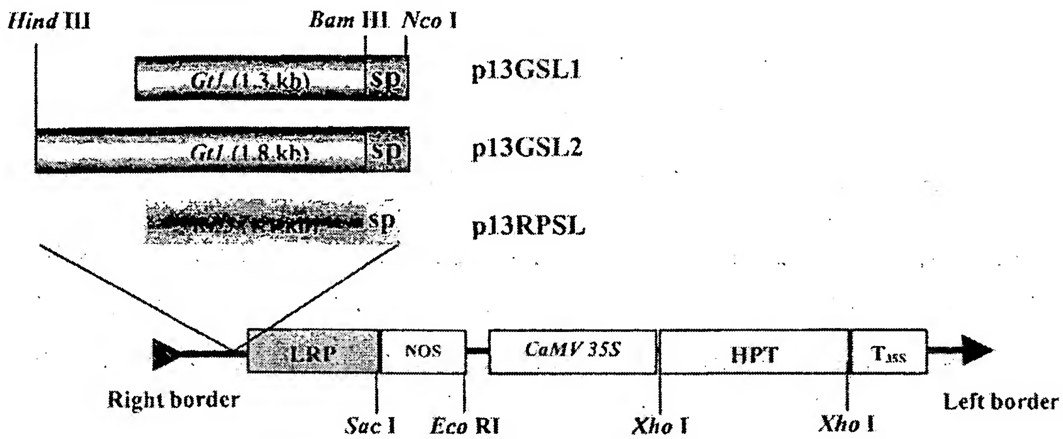


图 3.3 含 Gt1 或 RP5 信号肽编码序列(*SP*)与 LRP cDNA 融合基因双元载体的结构

**Fig. 3.3 The structure of binary vectors containing the LRP chimeric gene with the signal peptide coding sequences of Gt1 or RP5**

相应位点中，即构建成含 1.3 kb *Gt1* 启动子、72 bp *SP* 序列以及 LRP cDNA 的融合基因，其双元载体称为 p13GSL1（图 3.4）。最后，用 *Hind* III 和 *Bam* HI 酶切并回收质粒 pGt1(1.8)中 1.8 kb 的 *Gt1* 启动子序列，用其代替 p13GSL1 中 1.3 kb 的 *Gt1* 启动子片段，构建成另一个含 Gt1 信号肽编码序列和 LRP cDNA 融合基因的载体 p13GSL2（图 3.4）。

#### 3.2.5.4.3 含醇溶蛋白 RP5 信号肽编码序列 LRP 融合基因的构建

用 *Hind* III 和 *Nco* I 酶切并回收含 *PR5* 启动子序列及其信号肽序列的 *RPS* 片段（见第 2 章），将这一片段克隆进上述 3.2.5.1.2 中获得的载体 p13GSL1 的相应位点中，以取代 *Gt1* 启动子及其信号肽序列，形成含 *RP5* 启动子及其信号肽编码序列、LRP cDNA 的融合基因，相应的双元载体称为 p13RPSL（图 3.4）。

### 3.2.6 含 LRP 嵌合基因的农杆菌超双元载体的构建

#### 3.2.6.1 农杆菌超双元载体的构建

为了能利用农杆菌介导转化法高效地获得共转化水稻植株，又构建了一套超双元载体（Super binary vector），即在同一个双元载体上构建两个 T-DNA 区，

其中一个含有抗性选择标记基因，而另一个则只含有目的基因。利用该系统可期望借助于一个农杆菌将两个独立的 T-DNA 区导入同一个植株细胞中，并整合到不同染色体或同一染色体的不同位置上，从而可在分离后代中获得只含目的基因、无抗性选择标记基因（Selectable marker free, SMF）的转基因植株。

超双元载体的构建流程如图 3.5 所示。首先，根据双元载体 pCAMBIA1300 的核苷酸序列，设计了两个引物 $LBP_1$（5'-CAA<u>GCGGCCGC</u>GAGATCATCCGTGTTT-3'）和 $LBP_2$（5'-GTA<u>GAATTC</u>GACCGGATCTGTCGATCGA-3'），以便从该双元载体中扩增其 T-DNA 区的左边界序列（Left border）。在这两个引物的 5'端分别加接上了 *Not* I 和 *Eco* RI（已用下划线标出）酶切位点，其中 $LBP_2$ 位于左加界序列靠近 T-DNA 一侧。PCR 反应的条件为 95°C、5min；95°C、50sec，55°C、50sec，72°C、30sec，30 个循环；72°C、7min。495 bp 长的 PCR 产物经 *Not* I 和 *Eco* RI 酶切后克隆进中间载体 pBluescript $SK^-$中，经鉴定的阳性克隆称为 pBSK/LB。第二步，用 *Hind* III 和 *Eco* RI 双酶切双元载体 pCAMBIA1300，再用 Klenow 大片段补平并让其自连，以去除该双元载体中的多克隆位点，形成无多克隆位点的双元载体 pC130。第三步，用 *Sac* II 和 *Not* I 双酶切双元载体 pC130，回收并纯化 3.8 kb 的含 T-DNA 区的一个片段，将其克隆进第一步的载体 pBSK/LB 中，构建成载体 pBSK/LB/T-DNA。第四步，用 *Sph* I 和 *Not* I 双酶切载体 pBSK/LB/T-DNA，再用 Klenow 大片段补平并让其自连，以去除该质粒中位于 *Sph* I 和 *Not* I 之间的 DNA 序列，形成载体 pBSK/LB/T-DNA(M)。第五步，用 *Sac* II 和 *Eco* RI 双酶切载体 pBSK/LB/T-DNA(M)，回收并纯化 3.4 kb 长的含一个 T-DNA 区和一个左加界序列的片段，用这一片段取代原双元载体 pCAMBIA1300 或 pCAMBIA1301 中 *Sac* II 和 *Eco* RI 之间的序列（含 T-DNA 区左边界和潮霉素抗性基因），即构建成含两个 T-DNA 区的超双元载体 pSB130 和 pSB130/GUS（图 3.6）。

### 3.2.6.2 含 LRP 嵌合基因的超双元载体的构建

为便于利用上述超双元载体将 LRP 嵌合基因导入水稻中，又将图 3.3-A 中双元载体 p13GL2 和图 3.4 中双元载体 p13GSL2 上的 *Hind* III 和 *Eco* RI 片段分别克隆进了超双元载体 pSB130 和 pSB130/GUS 的多克隆位点中，构建了含 1.8 kb 长 *Gt1* 启动子、LRP cDNA 和 NOS 终止子序列的超双元载体 pSB130/GL2 和 pSB130/GUS/GL2，以及含 Gt1 信号肽编码序列和 LRP 融合基因的超双元载体 pSB130/GSL2（图 3.7）。

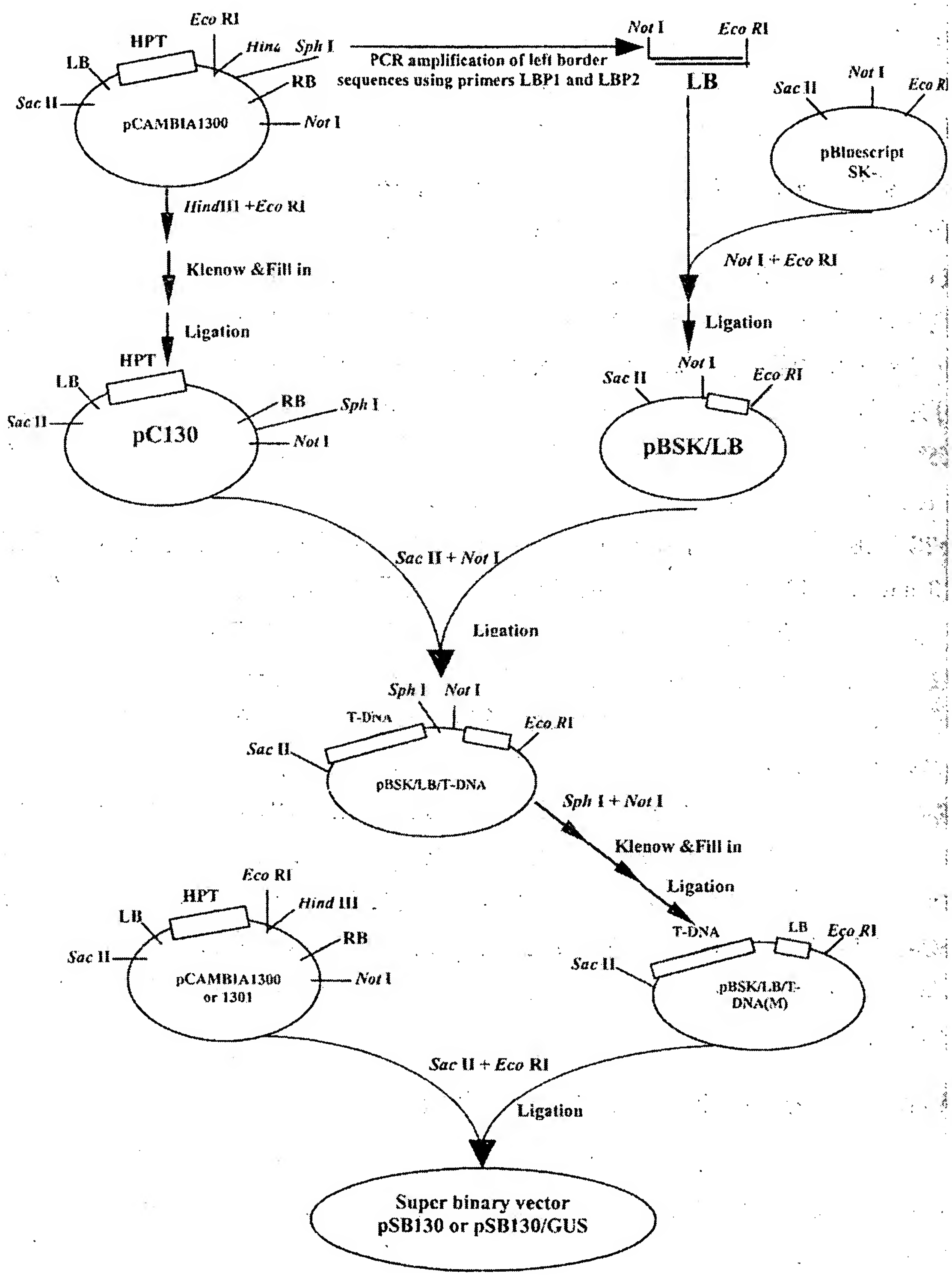


图 3.5 农杆菌超双元载体 pSB130 和 pSB130/GUS 的构建流程

Fig. 3.5 Construction of the super binary vectors pSB130 and pSB130/GUS

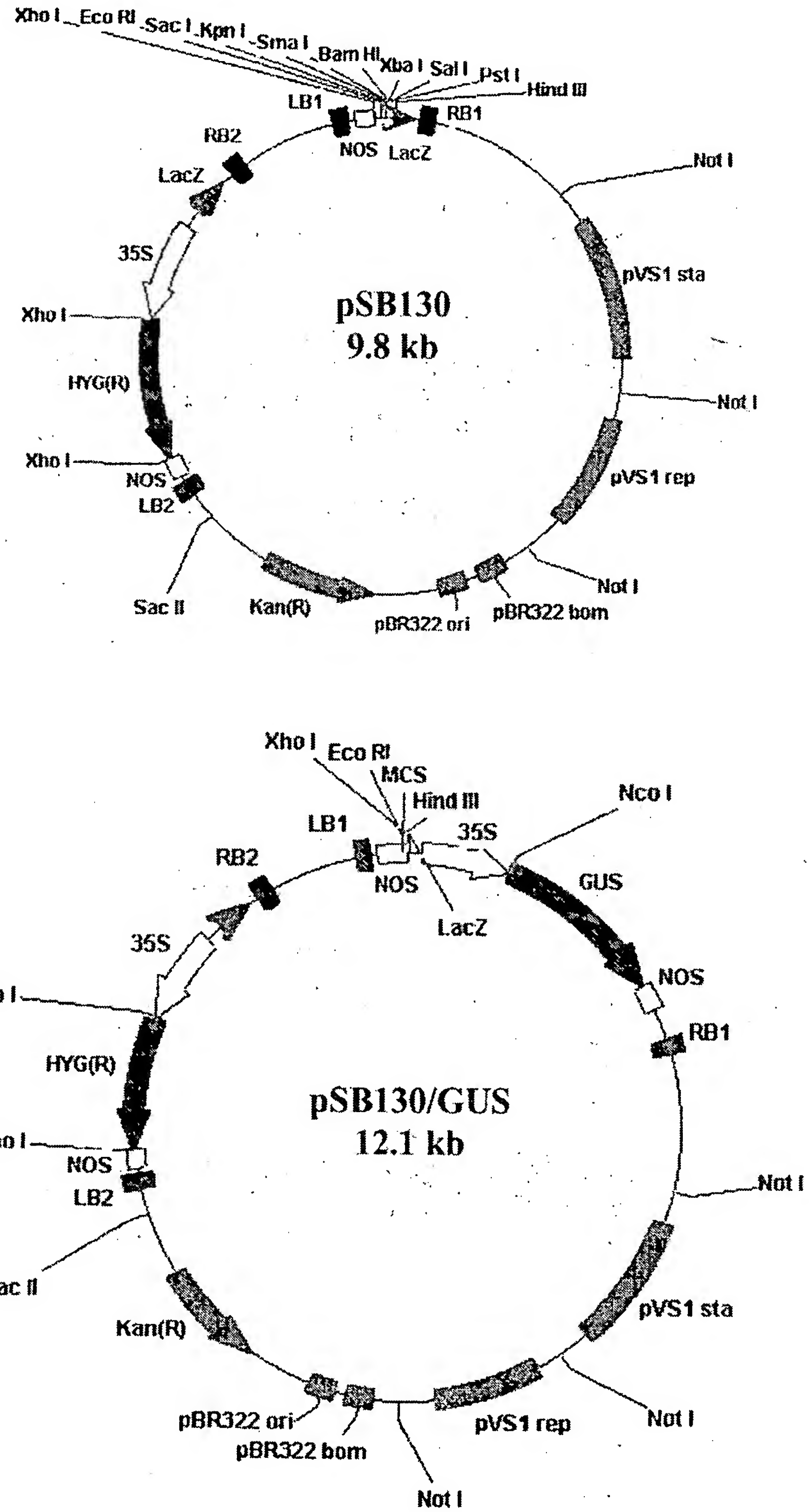


图3.6 农杆菌超双元载体 pSB130 和 pSB130/GUS 的结构

Fig. 3.6 The structure of super binary vectors pSB130 and pSB130/GUS

# 基因工程技术提高稻米赖氨酸含量



| | |
|---|---|
| 【英文题名】 | Genetically Engineering Rice for Increased Lysine |
| 【作者】 | 刘巧泉; |
| 【导师】 | 顾铭洪; 辛世文; |
| 【学位授予单位】 | 扬州大学; |
| 【学科专业名称】 | 作物栽培与耕作 |
| 【学位年度】 | 2002 |
| 【论文级别】 | 博士 |
| 【网络出版投稿人】 | 扬州大学 |
| 【网络出版投稿时间】 | 2003-12-09 |
| 【关键词】 | 转基因水稻; 赖氨酸; 富含赖氨酸含量蛋白; 水稻种子贮藏蛋白; 融合蛋白; 营养品质; 共转化法; |
| 【英文关键词】 | transgenic rice; lysine; lysine-rich protein; rice seed storage proteins; fusion protein; nutritional quality; co-transformation; |
| 【中文摘要】 | 植物是人类和牲畜所消耗的蛋白质的主要来源，但其营养品质往往不够完全。一般说，禾谷类作物种子蛋白质中的赖氨酸和色氨酸含量低，而豆类和蔬菜类蛋白质缺乏蛋氨酸和半胱氨基酸等含硫氨基酸。水稻是世界上最主要的粮食作物之一，稻米品质的优劣对人类健康具有重要的影响。稻米所含能量高，其中的贮藏蛋白易被消化吸收，但蛋白质含量较低，赖氨酸含量也缺乏，是稻米蛋白质中的第一限制必需氨基酸。所以，提高稻米中的必需氨基酸含量和蛋白质含量，平衡其营养品质，一直是遗传育种学家追求的目标之一。常规育种技术已在鉴别突变体以改良主要粮食作物蛋白质营养品质等方面获得了一些进展，但在水稻中却收效甚微。因此，寻求更为直接有效的途径去改良稻米的营养品质是非常必要的，分子生物技术的发展为改良稻米种子蛋白质营养品质提供了一条有效的技术路线。本研究即是要通过基因工程技术，在水稻种子胚乳中高效表达来自异源植物四棱豆的一个富含赖氨酸的蛋白质(Lysine-rich protein，LRP)，以提高稻米中的赖氨酸含量，最终达到改良稻米营养品质的目的。为此，重点开展了三个方面的研究工作，包括：(1)水稻主要贮藏蛋白基因启动子的分离及其功能鉴定，(2)LRP在转... |
| 【英文摘要】 | Plants are the primary source of all proteins consumed by humans and livestock. However, most plant proteins are nutritionally unbalanced, because they are deficient in certain essential amino acids. In general, cereal proteins are low in lysine and tryptophan while legume and most vegetable proteins are deficient in methionine and cysteine. Rice (Oryza sativa L.), one of the leading food crops and the staple food of over half the world's population, is a very good and relatively cheap source of energy ... |
| 【DOI】 | cnki:CDMD:11117.2.2002.2960 |
| 【相同导师文献】 | [1] 赵祥强.水稻半矮秆基因sdt2的鉴定、精细定位与候选基因筛选.扬州大学,2005<br>[2] 刘巧泉.基因工程技术提高稻米赖氨酸含量.扬州大学,2002<br>[3] 韩月澎.稻米淀粉合成相关基因对品质的影响及分支酶基因Sbe1、Sbe3克隆与分析.扬州大学,2004<br>[4] 陈秀花.转反义Sbe基因提高稻米直链淀粉含量的研究.扬州大学,2003 |

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本文参考文献的参考文献，进一步反映本文研究工作的背景和依据。

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